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(54) Title: OVERLYING ELECTRODE FOR ELECTROCHEMICAL MICROARRAYS

(57) Abstract: There is disclosed an improved electrochemical-based microarray biochip that is able to increase chip density by moving a counter-electrode out of a grid pattern of microarrays and into an overlaying position using thin film electrodes.

OVERLYING ELECTRODE FOR ELECTROCHEMICAL MICROARRAYS

Technical Field of the Invention

The present invention provides an improved electrochemical-based microarray device that is able to increase chip density by moving a counter-electrode out of a grid pattern of microarrays and into an overlaying position using thin film electrodes. Specifically, the overlaying electrode is preferably a thin film. In those embodiments where the overlaying electrode remains during detection, the overlaying electrode does not interfere with label (often fluorescent) detection nor does the overlaying electrode have non-specific binding properties.

Background of the Invention

In the world of microarrays, biological molecules (*e.g.*, oligonucleotides, polypeptides and the like) are placed onto surfaces at defined locations for potential binding with target samples of nucleotides or receptors. Microarrays are miniaturized arrays of biomolecules on a variety of platforms. Much of the initial focus for these microarrays have been in genomics with an emphasis of single nucleotide polymorphisms (SNPs) and genomic DNA detection/validation, gene expression, functional genomics and proteomics (Wilgenbus and Lichter, *J. Mol. Med.* 77:761, 1999; Ashfari et al., *Cancer Res.* 59:4759, 1999; Kurian et al., *J. Pathol.* 187:267, 1999; Hacia, *Nature Genetics* 21 suppl.:42, 1999; Hacia et al., *Mol. Psychiatry* 3:483, 1998; and Johnson, *Curr. Biol.* 26:R171, 1998).

There are, in general, three categories of microarrays (also called "biochips" and "DNA Arrays" and "Gene Chips" but this descriptive name has been attempted to be a trademark): (1) those spotted onto a solid surface (*i.e.*, usually silicon-based and most often a glass microscopic slide) with a computer –controlled printing device, (2) photolithographic techniques for *in situ* oligonucleotide synthesis (see, for example, Fodor U.S. Patent 5,445,934 and the additional patents that claim priority from this priority document), (3) electrochemical *in situ* synthesis based upon pH based removal of blocking chemical functional groups (see, for example, Montgomery U.S. Patent 6,093,302 the disclosure of which is incorporated by reference herein and Southern U.S. Patent 5,667,667), and (4) electric field attraction/repulsion of fully-formed oligonucleotides (see, for example, Hollis et al., U.S. Patent 5,653,939 and its duplicate Heller U.S. Patent 5,929,208). Only the first three basic techniques can form oligonucleotides *in situ*, that is, building each oligonucleotide, nucleotide-by-nucleotide, on the microarray surface without placing or attracting fully-formed oligonucleotides.

The electrochemistry platform (Montgomery U.S. Patent 6,093,302) provides a microarray based upon a semiconductor chip platform having a plurality of microelectrodes. This chip design uses Complimentary Metal Oxide Semiconductor (CMOS) technology to create high-density arrays of microelectrodes with parallel addressing for selecting and controlling individual microelectrodes within the array. The electrodes activated with current flow generate electrochemical reagents (particularly acidic protons) to alter the pH in a small,

defined "virtual flask" region or volume adjacent to the electrode. The microarray is coated with a porous matrix as a reaction layer material. Thickness and porosity of the material is carefully controlled and biomolecules are synthesized within volumes of the porous matrix whose pH has been altered through controlled diffusion of protons generated electrochemically and whose diffusion is limited by diffusion coefficients and the buffering capacities of solutions. However, in order to function properly, the microarray biochips using electrochemistry means for *in situ* synthesis has to alternate anodes and cathodes in the array in order to generated needed protons (acids) at the anodes so that the protons and other acidic electrochemically generated acidic reagents will cause an acid pH shift and remove a blocking group from a growing oligomer and are spaced such as to provide a more uniform electric field to generate electrochemical reagents. Therefore, only about 50% of the cells or electrode sites on a chip can be active sites for creation of biomolecules *in situ*. Therefore, there is a need in the art to increase the site densities of microarray biochips by moving cathodes out of the array surface. The present invention was made to meet this need and essentially double chip site densities.

Electrodes have been made from a variety of conductive materials including thin metallic oxides used as transparent conductors. For example, films of tin oxide doped with fluorine or of indium oxide doped with tin (*i.e.*, indium tin oxide) have been obtained by standard procedures, such as thermal evaporation, sputtering, or hydrolysis of metallic chlorides (spraying), or pyrolysis of organometallic compounds (chemical vapor deposition) (Manifacier, *Thin Solid Films* 90:297-308, 1982). Transparent and conductive layers of some metallic oxides, such as tin oxide or indium oxide, have been known for more than 50 years, have high stability, hardness and adherence to many substrates. They are deposited by a variety of techniques. For example, one technique involves cathode sputtering. Briefly, every sputtering process involves the creation of a gas plasma (often in argon) in a low-pressure chamber between a cathode, target holder, and the anode which is often used as the substrate holder. The discharge was set up by applying a voltage between the anode and the cathode. The flow of electrons from the cathode towards the anode ionizes the argon in the vicinity of the anode and these positive ions, in turn, bombard the target on the cathode. By momentum transfer they then eject particles that are deposited onto the substrate. Chemical vapor deposition, for example, involves gases introduced into a chamber where they react. The oxidizing agents are usually O_2, H_2O or even H_2O_2. The tin or indium compounds may be evaporated at relatively low temperature (about 100 °C) when organometallic compounds are used or at higher temperatures when chlorides are used. In a spraying method, a solution of $SnCl_4$ or $InCl_3$ in a mixture of alcohol and water is sprayed onto a heated substrate. However, despite the ease of spraying methods, in order to vaporize a solution, a relatively large separation of the nozzle and substrate is required. This leads to a relatively low efficiency and a high consumption of chemical in comparison with other techniques.

In addition, double-stranded DNA and DNA/RNA and RNA/RNA complexes in a double helical configuration are stable molecules that require aggressive conditions *in vitro* in order to separate complimentary strands of the nucleic acid. Commonly employed methods include, for example, heating the sample to at least 60 °C for about 10 min or use of an alkaline pH of about 11 or higher. Other methods include the use of helicase enzymes, such as Rep protein of *E. coli* that can catalyze the unwinding of the DNA in an unknown way, or binding proteins, such as the binding protein of 32-protein of *E. coli* phage T4, that can stabilize the single stranded form of DNA. The most common method for denaturation is heat or basic pH to produce a single stranded form of DNA for subsequent amplification cycles by common PCR techniques.

PCR techniques generally require a thermocycler that is able to alternatively heat and then cool a sample to denature and allow renaturation for nucleic acid samples around primer regions of a DNA backbone. In the case of microarray assays having oligonucleotide capture probes, DNA samples for analysis require a significant workup that is often done with a reagent kit. The DNA samples are first isolated and then denatured, flowed by amplification, such that amplified single-stranded samples are applied to microarrays with oligonucleotide capture probes as "content" (meaning the compilation of oligonucleotide sequences on a particular microarray). Sample preparation is done in solution test tubes or microtiter plates. Once completed, the "prepped" sample is applied to the microarray by a manual transfer with a pipette. However, there exists a need in the art to better automate and facilitate this sample preparation process by adding features to the microarray so that is also functions as a sample preparation biochip. The present invention further addresses this need with a feature that can both double site density in electrochemically-based microarrays and provide resistive heat control to automate denaturation heating and renaturation cooling of a sample already applied to the test microarray. This has resulted in a simplification of handling and reducing the hardware required for manufacturing microarrays and for synthesizing oligomer content.

Summary of the Invention

The present invention provides a blank biochip for synthesis of oligomers at selected sites comprising:

(a) a semiconductor chip base having a top surface and a bottom surface, and having a plurality of cells arranged in a pattern, wherein each cell comprises an electrode and switching circuitry able to control current or voltage flow to the electrode;

(b) a porous membrane layer overlaying the top surface of the semiconductor chip;

(c) a spacing layer overlaying the porous membrane layer so that the porous membrane acts as a first wall of a fluidics channel, wherein the spacing layer is capable of acting as the fluidics channel; and

(d) an overlaying electrode comprising a conductive film layered onto a solid surface, wherein the conductive film acts as a second wall of the fluidics channel.

Preferably, the fluidics channel is in communication with an inlet means and an outlet means for fluid flow within the spacing layer. Preferably, the overlaying electrode acts as a cathode and one or a plurality of electrodes in the semiconductor chip acts as anodes so that when current is flowing, the anodes generate protons to affect pH of a solution in a regional volume of the porous matrix defined by the geometry of the electrode as a base and top of the volume with walls extending between each two-dimensional structure. Most preferably, the electrode is in the form of a circle and the volume defines a cylinder. Most preferably, the electrode is in the form of a square and the volume defines a cube. Preferably, the overlaying electrode extends to an array covering the entire top surface of the cells in the semiconductor chip. Preferably, the overlaying electrode is composed of a conductive film that is transparent or translucent to allow passage of at least 50% of electromagnetic radiation in the visible and surrounding spectra. Most preferably, the overlaying electrode is composed of a metallic oxide doped with tin or aluminum. Most preferably, the overlaying electrode is composed of a conductive metal or alloy thereof, platinum, or a metallic oxide selected from the group consisting of tin-doped indium oxide (ITO), aluminum-doped zinc oxide (AZO), and combinations thereof.

The present invention further provides a microarray having a plurality of different oligonucleotides synthesized *in situ* at selected sites comprising:

(a) a semiconductor chip base having a top surface and a bottom surface, and having a plurality of cells arranged in a pattern, wherein each cell comprises an electrode and switching circuitry able to control current flow to the electrode;

(b) a porous membrane layer overlaying the top surface of the semiconductor chip;

(c) a spacing layer overlaying the porous membrane layer so that the porous membrane acts as a first wall of a fluidics channel, wherein the spacing layer is capable of acting as the fluidics channel; and

(d) an overlaying electrode comprising a conductive film layered onto a solid surface, wherein the conductive film acts as a second wall of the fluidics channel.

Preferably, the fluidics channel is in communication with an inlet means and an outlet means for fluid flow within the spacing layer. Preferably, the overlaying electrode acts as a cathode and one or a plurality of electrodes in the semiconductor chip acts as anodes so that when current is flowing, the anodes generate protons to affect pH of a solution in a regional volume of the porous matrix defined by the geometry of the electrode as a base and top of the volume with walls extending between each two-dimensional structure. Most preferably, the electrode is in the form of a circle and the volume defines a cylinder. Most preferably, the electrode is in the form of a square and the volume defines a cube. Preferably, the overlaying electrode extends to a uniform covering of the entire top surface of the cells in the semiconductor chip. Preferably, the overlaying electrode is composed of a conductive film that is transparent or translucent to allow passage of at least 50% of electromagnetic radiation in the visible and surrounding spectra. Most preferably, the overlaying electrode is composed

of a metallic oxide doped with tin or aluminum. Most preferably, the overlaying electrode is composed of a metallic oxide selected from the group consisting of tin-doped indium oxide (ITO), aluminum-doped zinc oxide (AZO), and combinations thereof.

The present invention further provides a process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions, comprising:

(a) providing a microarray chip having a plurality of different oligonucleotide capture probes, wherein the microarray chip comprises:

(i) a semiconductor chip base having a top surface and a bottom surface, and having a plurality of cells arranged in a pattern, wherein each cell comprises an electrode and switching circuitry able to control current flow to the electrode;

(ii) a porous membrane layer overlaying the top surface of the semiconductor chip;

(iii) a spacing layer overlaying the porous membrane layer so that the porous membrane acts as a first wall of a fluidics channel, wherein the spacing layer is capable of acting as the fluidics channel; and

(iv) an overlaying electrode comprising a conductive film layered onto a solid surface, wherein the conductive film acts as a second wall of the fluidics channel and is wired to a current or voltage source; and

(b) adding a DNA sample for analysis in a liquid to the spacing layer; and

(b) applying a resistive current across the overlaying electrode to increase the temperature of the liquid in the spacing layer to control a hybridization reaction, whereby increasing the temperature favors denaturation of double stranded nucleic acids and lower temperatures favors renaturation or hybridization of nucleic acids.

Preferably, the fluidics channel is in communication with an inlet means and an outlet means for fluid flow within the spacing layer. Preferably, the overlaying electrode acts as a cathode and one or a plurality of electrodes in the semiconductor chip acts as anodes so that when current is flowing, the anodes generate protons to affect pH of a solution in a regional volume of the porous matrix defined by the geometry of the electrode as a base and top of the volume with walls extending between each two-dimensional structure. Most preferably, the electrode is in the form of a circle and the volume defines a cylinder. Most preferably, the electrode is in the form of a square and the volume defines a cube. Preferably, the overlaying electrode extends to a uniform covering of the entire top surface of the cells in the semiconductor chip. Preferably, the overlaying electrode is composed of a conductive film that is transparent or translucent to allow passage of at least 50% of electromagnetic radiation in the visible and surrounding spectra. Most preferably, the overlaying electrode is composed of a metallic oxide doped with tin or aluminum. Most preferably, the overlaying electrode is composed of a a conductive metal, platinum, or a metallic oxide selected from the group

consisting of tin-doped indium oxide (ITO), aluminum-doped zinc oxide (AZO), and combinations thereof. Preferably, the microarray further comprises a temperature sensor wired in a feedback look to the overlaying electrode to regulate temperature.

Brief Description of the Drawings

Figure 1 shows a cross-section schematic of an overlaying cathode over a microarray device having a plurality of electrodes arranged in a grid pattern.

Figure 2 shows the results of an expression assay using the inventive microarray device, as detailed in Example 1. Specifically, average 35 mer oligonucleotide capture probes were synthesized on an electrode-containing microarray device having a common overlaying cathode according to the present invention. The overlaying cathode was made from an ITO film. The sample used was placental cRNA using a fluorescent label (Cy5).

Figure 3 shows a patterned overlaying electrode scheme to function as a resistive heater.

Detailed Description of the Invention

The present invention provides a blank microarray biochip that is able to double the number of sites available for *in situ* synthesis by electrochemical means in the same area chip with the same size of unit cells and electrodes in each unit cell. The present invention is an improvement over the original microarray designs for *in situ* electrochemical synthesis of oligomers (*e.g.*, oligonucleotides, polypeptides and small molecules) in that it moves the needed counter electrode to an overlaying electrode and away from an alternative electrode-counter electrode grid pattern. The net result of this step is to effectively double the number of active sites available for electrochemical *in situ* synthesis on a microarray grid. A preferred microarray for *in situ* synthesis of oligomers is described in United States Patent 6,093,302, the disclosure of which is incorporated by reference herein. The '302 Patent shows a microarray semiconductor chip having a grid of cells, wherein each cell has an electrode (Figure 16 of the '302 Patent shows the electrode in a circular shape) and switching circuitry such that each electrode is separately addressable and can be a cathode or an anode, wherein the anode (that generates protons and other electrochemical reagents) is often called the electrode and the cathode the counter electrode because *in situ* synthesis based upon acid pH shifts occurs at the anode. However, basic shifts with appropriate base-cleavable blocking groups can occur at a cathode with an anode being the counter electrode. In addition, Southern U.S. Patent 5,667,667 shows alternating rows or columns of anodes and cathodes with DNA synthesis occurring on an overlaying glass slide or "surface." Therefore, Southern shows a different geometry whose elements are located in different places.

Semiconductor chips having microarray structures with separately addressable electrodes have been fabricated. The density per unit area of chip surface or the number of cells that can be packed into a unit area is a function of the ever-decreasing size of the features

that can be laid down. Therefore, the microarrays having separately addressable multiple electrodes for *in situ* synthesis, preferably electrochemical synthesis, have been made and continue to become denser as semiconductor technology allows for the fabrication of smaller and smaller feature sizes.

Making An Overlaying Electrode

The overlaying electrode element is made of a conductive thin film layered onto a solid substrate. Preferably, the solid substrate is glass or another silica-based material. Preferred conductive films are platinum, ITO (tin-doped indium oxide), and AZO (aluminum-doped zinc oxide). Only ITO and AZO are also transparent. Preferably, the conductive material was coated onto the substrate by standard techniques. A pulsed laser deposition process for ITO and AZO was described in Kim et al. (SPIE 3797:290, 1999). Briefly, a KrF excimer laser (Lambda Physics LPX 305 at 248 nm and pulse duration of 30 ns) was used to deposit conductive films onto glass substrates. The laser was operated at a pulse rate of 10 Hz and the laser beam quality was improved by passing it through a spatial filter. The laser beam was focused through a 50 cm focal length lens onto a rotating target at a 45° angle of incidence. The energy density of the laser beam at the target surface was maintained at 2 J/cm^2. The target substrate distance was 4.7 cm. The laser beam was rastered across the surface of the target with a computer-controlled mirror while the target was rotated. This process produced uniform films over a 1.5 cm by 1.5 cm square surface with a thickness variation of less than 10%.

ITO targets have been prepared from In_2O_3 and SnO_2 powders. AZO has been prepared from ZnO and Al_2O_3 powders. The powders can be mixed in a mixer or shaker and pressed into pellets and then scintered.

The overlaying cathode is made, for example with ITO as a film, by depositing by electron beam evaporation. A glass substrate (50mm x 75mm x 1.1mm) was coated on one side by ITO deposited by electron beam evaporation (Thin Film Technology, Inc. Buelton, CA). The thin film has a resistance of between 17 and 20 ohms/square. The thickness of the film was about 1700Å.

In order to form an electrode, TiW and Au metal was deposited sequentially on the glass to form electrical contacts to the ITO film. The deposition system was an MRC Model 822 sputter deposition system (Washington Technology Center Microfabrication Laboratory, Seattle, WA). The TiW film was deposited for 3 min at 200W of RF power and 8mT of argon to an approximate thickness of 300Å. The Au film was deposited at 200W of RF power and 8mT of argon for 10 minutes (5 sputter 5 min rest/cool down followed by final 5 min deposition) to an approximate thickness of 3000Å. The areas to be free of metal on the glass slide were masked prior to deposition using "medium tack" blue dicing tape (Semiconductor Equipment Corp. Moorpark, CA). After deposition, the tape was removed by soaking in a solvent solution of acetone followed by isopropyl alcohol. The glass was then cut to a size of 5.1 mm by 26.0 mm to fit over a microarray chip.

In a preferred embodiment a polymeric coating layer is placed over the thin film overlaying electrode. The polymeric coating layer functions to prevent non-specific bonding of biologic molecules and target samples to the overlaying electrode to create "noise" during analysis of binding of target molecules to specific binding sites on the microarray. Examples of polymeric materials are hydrophilic polymers. Preferred hydrophilic polymers are selected from the group consisting of PEG (polyethylene glycol having a molecular weight from about 600 daltons to about 6000 daltons), oligoethylene glycol, polyhydroxyethyl methacrylate, polyvinylalcohol, phospholipids, and combinations thereof. Preferred techniques for depositing a polymeric layer, preferably a hydrophilic polymeric layer, include techniques such as radiation grafting, radio frequency deposition, chemical grafting, co-polymer adsorption and chemisorption.

A CMOS (Complementary Metal-Oxide Semiconductor) process was used to fabricate the electronic component of the microarray device. The assembly of the microarray biochip starts by having a die diced, attached and wire bonded onto a PGA (Pin Grid Array) ceramic package (Corwil, Inc., San Jose, CA). A porous reaction layer is then deposited and cured onto the chip. The inventive overlaying electrode is configured to have the film placed at a spacing of from about 2500 microns (0.01 inches) to about 25 microns (0.001 inches) above the surface of the porous reaction layer or microarray device. Preferably, the spacing is approximately 0.004 inches. In this implementation, a spacer film is used to establish the gap or spacing. For example, a polyimide film of about 100 μm thickness is used to establish the spacing. The spacer film is adhered to the microarray using an epoxy paste, however, care should be taken to avoid contact of the epoxy resin with the porous reaction layer so that the porous reaction layer is not contaminated. The chip is assembled and then baked to cure the epoxy. The microarray chip is now considered a "blank chip" ready for oligomer content to be added. Finally, a flex circuit is soldered to form an electrical contact to the overlaying electrode.

The inventive microarray design, having content synthesized *in situ*, further allows for an electric field to be placed across the spacing layer during assay hybridization. The electric field will better align the oligonucleotide capture probes previously synthesized due to a directional electric field. The oligonucleotide alignment provides for better access of the single stranded nucleic acid from the assay sample to the oligonucleotide capture probes on multiple sites on the microarray within the porous reaction layer.

Example 1

This example provides the results of a gene expression assay with a known target mRNA sample using a higher density microarray having an inventive overlaying electrode (cathode) structure wherein the cathode is a film of ITO made as described herein. Briefly, a microarray chip having an overlaying electrode was manufactured as described herein for the overlaying electrode and as described in Montgomery U.S. Patent 6,093,302 (the disclosure of which is incorporated by reference herein) for the CMOS microarray having a plurality of

microelectrodes. The microarray was synthesized with oligonucleotide content with all sites having the same 15-mer oligonucleotide binding probe (5' TACGCCACCAGCTCC 3'). The 3' end of the capture probe was bound to the chip. The chip, with synthesized capture probes, was swelled by dipping in a solution of 6X SSPE and washed. A solution of oligonucleotide (5 nM) having a fluorescent probe (Texas Red) (and the sequence 3' ATGCGGTGGTCGAGG) was placed over the chip and incubated (for hybridization) at 40 °C for 30 min. The chip was washed with a buffer solution and then allowed to dry. The surfaced was imaged using proper wavelengths of light for excitation and emission for the fluorescent dye. The image (Figure 3 showed that each cell showed hybridization with the target. Therefore, these data showed that the inventive overlaying electrode allowed for proper *in situ* electrochemical synthesis of oligonucleotide capture probes on the surface of a CMOS chip allowing all cells to be used as test sites and not just 50% of the cells due to the need for counter-electrodes.

We claim:

1. A blank microarray for synthesis of oligomers at selected sites comprising:

(a) a semiconductor chip base having a top surface and a bottom surface, and having a plurality of cells arranged in a pattern, wherein each cell comprises an electrode and switching circuitry able to control current flow to the electrode;

(b) a porous membrane layer overlaying the top surface of the semiconductor chip;

(c) a spacing layer overlaying the porous membrane layer so that the porous membrane acts as a first wall of a fluidics channel, wherein the spacing layer is capable of acting as the fluidics channel; and

(d) an overlaying electrode comprising a conductive film layered onto a solid surface, wherein the conductive film acts as a second wall of the fluidics channel.

2. A microarray having a plurality of different oligonucleotides synthesized *in situ* at selected sites comprising:

(a) a semiconductor chip base having a top surface and a bottom surface, and having a plurality of cells arranged in a pattern, wherein each cell comprises an electrode and switching circuitry able to control current flow to the electrode;

(b) a porous membrane layer overlaying the top surface of the semiconductor chip;

(c) a spacing layer overlaying the porous membrane layer so that the porous membrane acts as a first wall of a fluidics channel, wherein the spacing layer is capable of acting as the fluidics channel; and

(d) an overlaying electrode comprising a conductive film layered onto a solid surface, wherein the conductive film acts as a second wall of the fluidics channel.

3. The microarray of claims 1or 2, wherein the fluidics channel is in communication with an inlet means and an outlet means for fluid flow within the spacing layer.

4. The microarray of claims 1or 2, wherein the overlaying electrode acts as a cathode and one or a plurality of electrodes in the semiconductor chip acts as anodes so that when current is flowing, the anodes generate protons to affect pH of a solution in a regional volume of the porous matrix defined by the geometry of the electrode as a base and top of the volume with walls extending between each two-dimensional structure.

5. The microarray of claim 4, wherein the electrode is in the form of a circle and the volume defines a cylinder.

6. The microarray of claim 4, wherein the electrode is in the form of a square and the volume defines a cube.

7. The microarray of claims 1or 2, wherein the overlaying electrode extends to a uniform covering the entire top surface of the cells in the semiconductor chip.

8. The microarray of claims 1or 2, wherein the overlaying electrode is composed of a conductive film that is transparent or translucent to allow passage of at least 50% of electromagnetic radiation in the visible and surrounding spectra.

9. The blank biochip for synthesis of oligomers at selected sites of claim 7, wherein the overlaying electrode is composed of platinum or a metallic oxide doped with tin or aluminum.

10. The microarray of claim 9, wherein the overlaying electrode is composed of a metallic oxide selected from the group consisting of tin-doped indium oxide (ITO), aluminum-doped zinc oxide (AZO), and combinations thereof.

10. A process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions, comprising:

(a) providing a microarray chip having a plurality of different oligonucleotide capture probes, wherein the microarray chip comprises:

(i) a semiconductor chip base having a top surface and a bottom surface, and having a plurality of cells arranged in a pattern, wherein each cell comprises an electrode and switching circuitry able to control current flow to the electrode;

(ii) a porous membrane layer overlaying the top surface of the semiconductor chip;

(iii) a spacing layer overlaying the porous membrane layer so that the porous membrane acts as a first wall of a fluidics channel, wherein the spacing layer is capable of acting as the fluidics channel; and

(iv) an overlaying electrode comprising a conductive film layered onto a solid surface, wherein the conductive film acts as a second wall of the fluidics channel and is wired to a current or voltage source; and

(b) adding a DNA sample for analysis in a liquid to the spacing layer; and

(b) applying a resistive current across the overlaying electrode to increase the temperature of the liquid in the spacing layer to control a hybridization reaction, whereby increasing the temperature favors denaturation of double stranded nucleic acids and lower temperatures favors renaturation or hybridization of nucleic acids.

11. The process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions of claim 10, wherein the fluidics channel is in communication with an inlet means and an outlet means for fluid flow within the spacing layer.

12. The process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions of claim 10, wherein the overlaying electrode acts as a cathode and one or a plurality of electrodes in the semiconductor chip acts as anodes so that when current is flowing, the anodes generate protons to affect pH of a solution in a regional volume of the porous matrix defined by the geometry of the electrode as a base and top of the volume with walls extending between each two-dimensional structure.

13. The process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions of claim 12, wherein the electrode is in the form of a circle and the volume defines a cylinder.

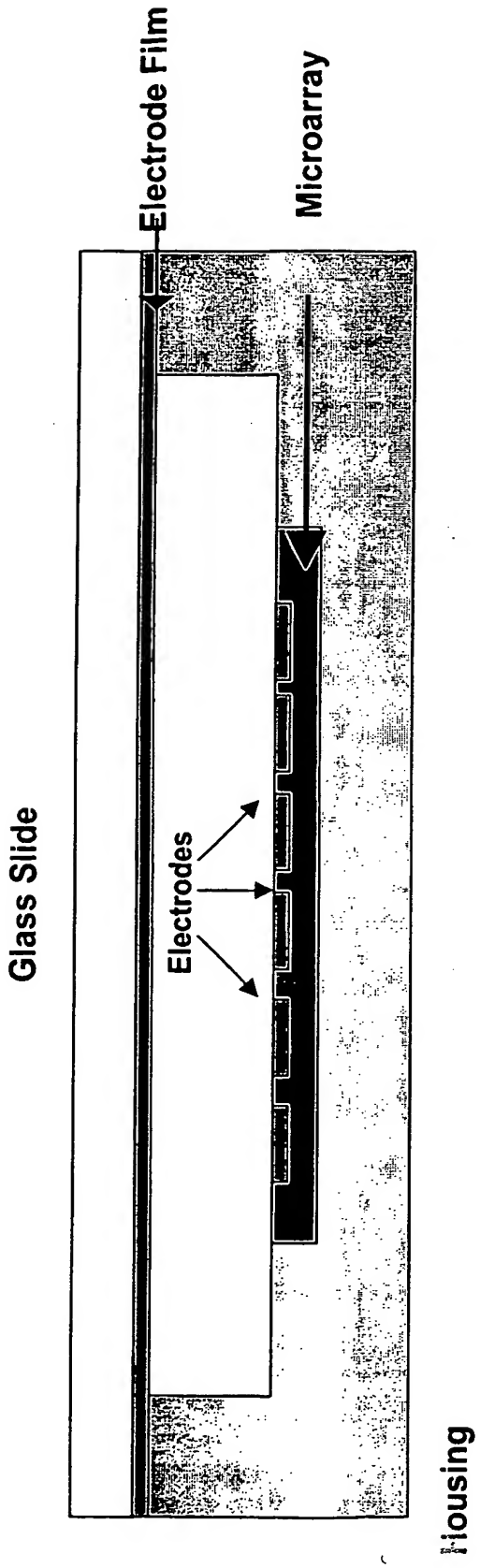
14. The process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions of claim 12, wherein the electrode is in the form of a square and the volume defines a cube.

15. The process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions of claim 10, wherein the overlaying electrode extends to an uniform covering of the entire top surface of the cells in the semiconductor chip.

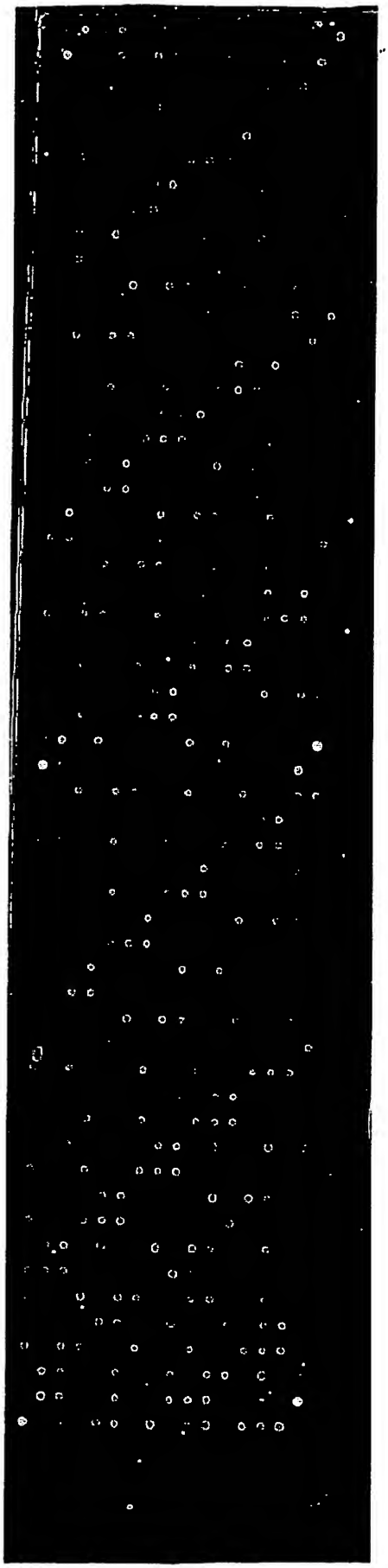
16. The process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions of claim 10, wherein the overlaying electrode is composed of a conductive film that is transparent or translucent to allow passage of at least 50% of electromagnetic radiation in the visible and surrounding spectra.

17. The process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions of claim 16, wherein the overlaying electrode is composed of a metallic oxide doped with tin or aluminum.

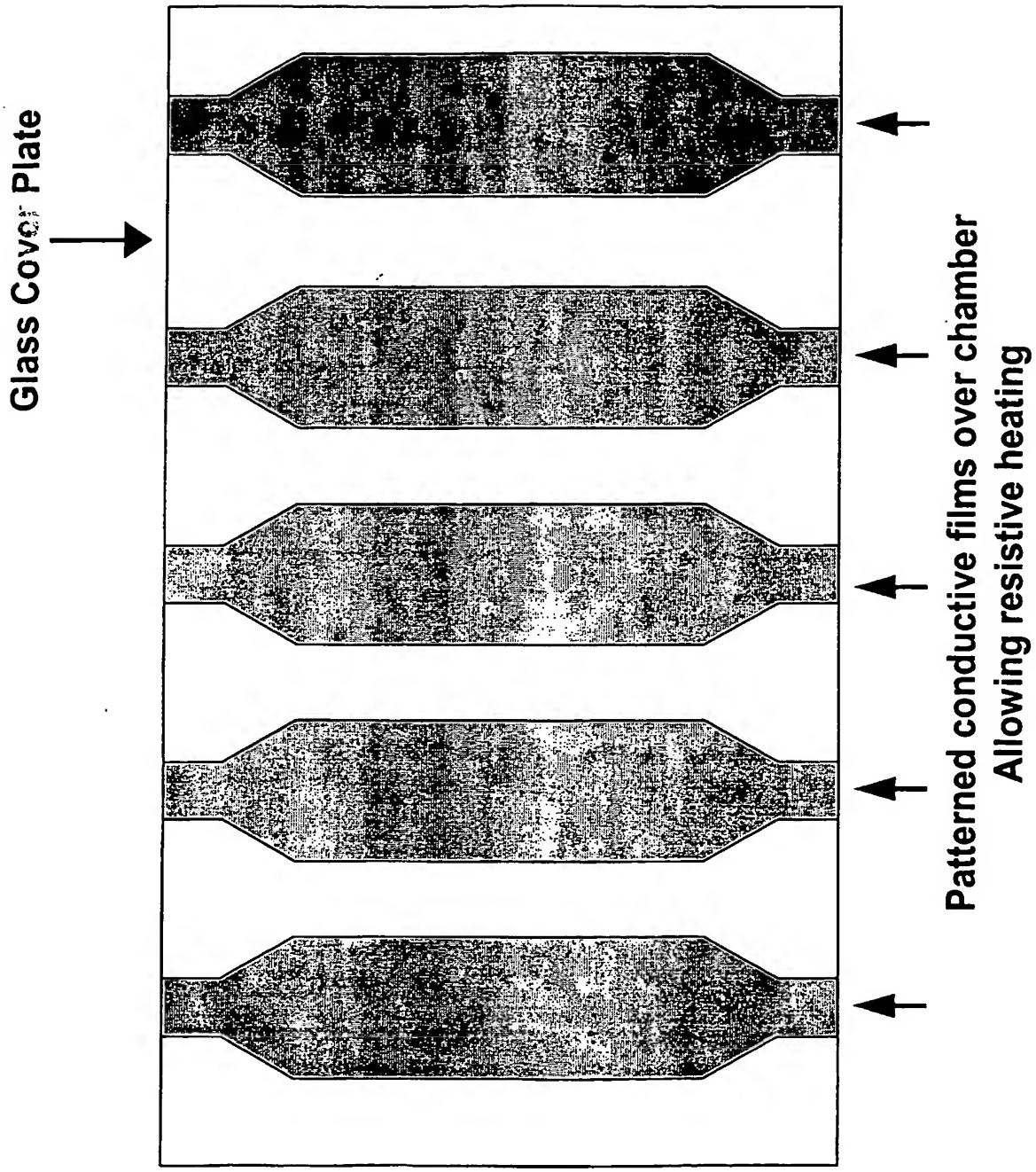
18. The process for controlling temperature of a microarray having a plurality of different oligonucleotides capture probes at selected sites for affecting denaturation of double stranded oligonucleotides and hybridization conditions of claim 17, wherein the overlaying electrode is composed of platinum or a metallic oxide selected from the group consisting of tin-doped indium oxide (ITO), aluminum-doped zinc oxide (AZO), and combinations thereof.



35 mers probes synthesized with common cathode



AR 1K sequence with Lambdas in both channels and cy5 placental cRNA



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/10669

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 27/26, 33/00, 21/00; C12Q 1/68; C12M 1/36; C12N 11/16; C07H 21/00
US CL : 422/68.1, 82.01; 435/283.1, 287.1, 287.2, 287.8; 536/25.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 422/68.1, 82.01; 435/283.1, 287.1, 287.2, 287.8; 536/25.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,165,335 A (LENNOX et al) 26 December 2000 (26.12.2000), column 11 and Fig. 6.	1-18
Y	US 6,093,302 A (MONTGOMERY) 25 July 2000 (25.07.2000), columns 13, 15, 26 and 27.	1-18
Y	US 6,093,370 A (YASUDA et al) 25 July 2000 (25. 07.2000) column 9 and 7-18 and Fig.9 and 24-25.	1-18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 June 2002 (13.06.2002)	03 JUL 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer BJ Forman Telephone No. (703) 308-0196

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